REMARKS

This response is due on March 29, 2004. Applicants file herewith a Request for Continued Examination. Applicants request that both the instant response and the Request be made of record and the claims be reconsidered for allowance in view of this response.

I. Status of the Claims

Claims 57-96, 100, 102 and 104-114 are pending in the instant application and stand variously rejected under 35 U.S.C. §102(b) and 35 U.S.C. §103(a). Applicants respectfully traverse the rejections and request reconsideration in light of the above amendments and the following remarks.

II. Rejections of the Methods Claims Under 35 U.S.C. §102(b) Should be Withdrawn

Claims 57 and 96 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by the disclosure of Koster (PCT International Publication No. WO 96/29431; Sept. 26, 1996). Applicants respectfully disagree.

As an initial matter, Applicants believe that the rejections being articulated against the instant claims stem from a misunderstanding or confusion of the patentable methods of sequencing that are being claimed herein with methods of detecting nucleic acids as provided in the cited art. In an effort to reduce the issues being discussed in the instant case and to resolve confusion relating to the methods described herein, Applicants will address the kit claims, which also were rejected, separately in Section IV below.

Referring now to the rejection of the methods of the present claims, Applicants initially reiterate that the claims of the present invention are directed to "a method of sequencing" a given target nucleic acid. The term "sequencing" (or "re-sequencing," where used) is used to indicate that the nucleotide sequence of the target nucleic acid is determined from a non-ordered set of fragments. These techniques provide an alternative to the conventional sequencing techniques available in the art. The techniques of the present invention use a "combination of several different mass spectra, obtained after complementary

digestion reactions" (page 4, lines 28-29). Thus, in the presently claimed invention, the products of multiple cleavage reactions of a target nucleic acid are subjected to mass spectrometry. Subsequently, the information from the various mass spectra is integrated to result in an unambiguous determination of sequence showing the nucleic acid residue at each position of the target nucleic acid, *i.e.*, the combination of mass shifts and/or changes in cleavage pattern observed in the mass spectra results in the unequivocal determination of the sequence (variations). Thus, the independent claim 57 embraces a technique that not only detects a mutation but, additionally, provide the identity of the target sequence in terms of its structure and thus allows one to determine the nature of the mutation, as well as, its location within the target nucleic acid sequence.

The methods claimed in the present invention have a number of steps that involve taking a target sequence and (1) subjecting it to at least two cleavage reactions to generate a non-ordered set of fragments; (2) analyzing the non-ordered fragments by mass spectrometry and (3) assembling the sequence of the target nucleic acid by performing a systematic computational analysis on the resultant mass spectra as described in the specification, to unambiguously determine the sequence of the target nucleic acid. These steps reveal the identity of the target sequence and not merely the relative presence or absence of a mutation. Furthermore, it is an express requirement of the claims herein that two or more cleavage reactions be used and that these reactions produce a set of fragments.

By contrast to the claims of the present invention and contrary to the assertions of the Examiner, the disclosure of Koster is only ***directed to detecting the presence of a mutation/variation***. For example, the abstract of Koster indicates that it is directed to "mass spectrometer based processes for detecting a particular nucleic acid sequence in a biological sample." The Summary of the Invention of the Koster document at page 4 teaches that it is directed to "detecting a particular nucleic acid sequence in a biological sample" and that this detection can be used to diagnose a given trait. Various "embodiments" are described, but all of these embodiments are based on determining the presence or absence of a known, reference sequence. None of the teachings of Koster demonstrate the use of mass spectrometry of non-ordered fragments, generated from multiple cleavage reactions, to deduce the sequence of a given target nucleic acid.

The Examiner specifically pointed to page 6, lines 8-14 and Figure 7B, Figure 8 and page 24, lines 1-12 as teaching multiple cleavage reactions as in step (b) of claim 57. Nevertheless, each of these teachings fails to describe a method of sequencing (i.e., assembling a sequence) of a target nucleic acid from a non-ordered set of fragments. The techniques described in Koster are all related to detecting the presence or absence of a nucleic acid. The text at page 24, lines 1-11 of Koster states that

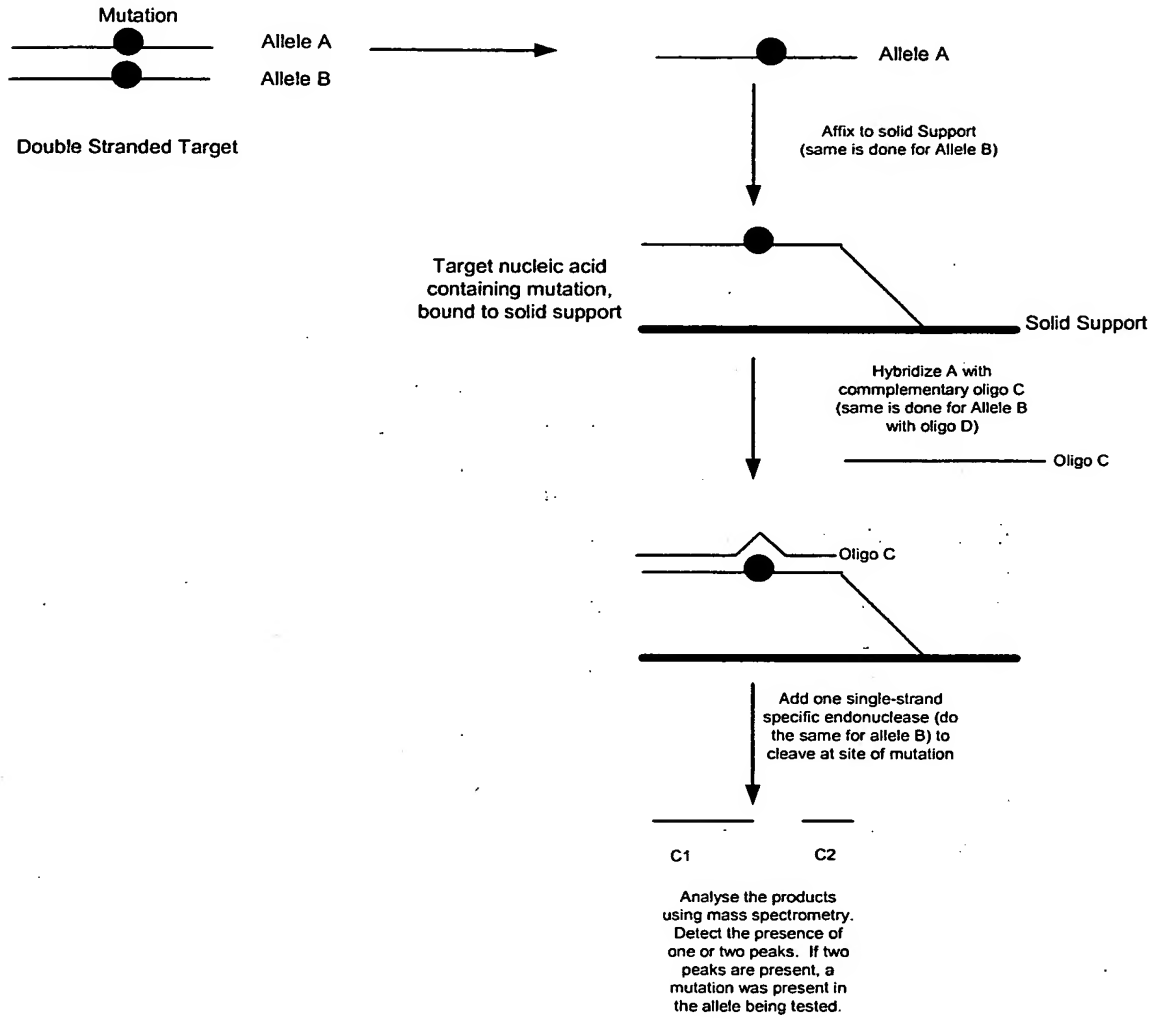
> "this invention makes use of the known sequence information of the target sequence and known mutation sites. Although new mutations can also be ***detected.*** For example as shown in FIGURE 8, transcription of a nucleic acid molecule obtained from a biological sample can be specifically digested using one or more nucleases and the fragments captured on a solid support carrying the complementary nucleic acid sequences. Detection of hybridization and the molecular weights of the captured target sequences provide information on whether and where a mutation is present. Alternatively, DNA can be cleaved by one or more specific endonucleases to form a mixture of fragments. ***Comparison of the molecular weights between wildtype and mutant fragment mixtures results in mutation detection.*** " ***(Emphases added)***

The legend for Figure 8, at page 9, lines 23-31 of Koster states that it is:

> A diagram showing how both strands of a target DNA can be prepared ***for detection*** using transcription vectors having two promoters at opposite locations (e.g., the SP6 and the T7 promoter). This format is particularly useful for detecting heterozygous target detection sites (TDS). Employing SP6 or the T7 polymerase both strands could be transcribed separately or simultaneously. Both RNAs can be specifically captured and simultaneously detected using an appropriately mass-differentiated detector oligonucleotides. This can be accomplished either directly in solution or by parallel processing of many target sequences on an ordered array of specifically immobilized capturing sequences."

It is quite clear from the disclosure of WO 96/29431 that Koster is directed to merely providing a mass spectrometry-based detection of a mutation in a given sequence without actually sequencing the entire sequence from a series of non-ordered fragments generated through multiple cleavage reactions. This is in contrast to the method of the present invention which provides the sequence identity of the chain of nucleotides using a

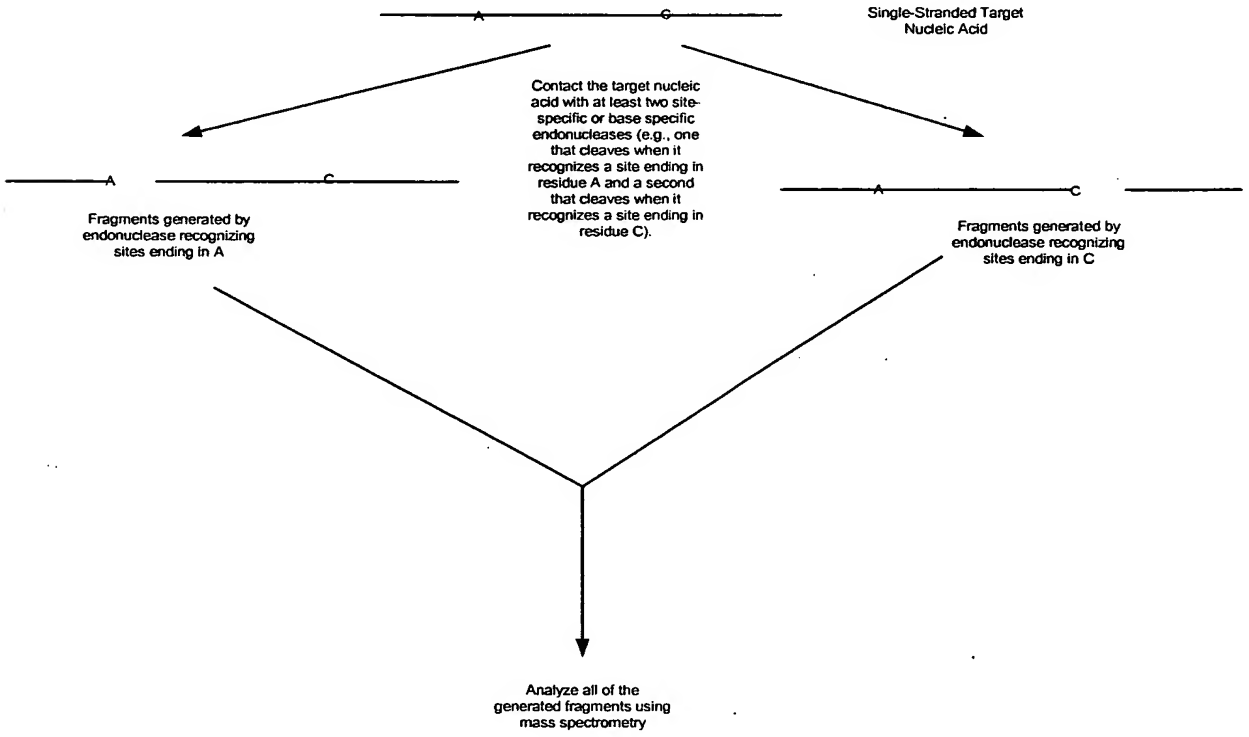
non-ordered set of fragments generated by two or more cleavage reactions. The method of Koster is described in Figure 7B and may further be illustrated as follows:



While both strands A and B are analyzed by digestion with a single-strand specific endonuclease, only ***one endonuclease reaction*** is conducted on each strand. If there is a mutation present on the strand that is being analyzed, that one endonuclease reaction will produce two fragments, a C1 fragment and a C2 fragment, generated from either side of the cleaved mutation site. A mass spectrometry analysis of oligonucleotide C is compared to the results of the endonuclease reaction to determine whether the sequence being analyzed contained a mutation or not. If there was is a mutation present, two peaks are revealed by mass spectrometry analysis; if a mutation is not present, then only the one peak associated

with oligonucleotide C is seen. At no time during this analytical analysis is the identity of the actual residue present at the given site revealed.

By contrast, the present application employs at least two separate endonucleases to interrogate the sequence of a given target nucleic acid. This can be depicted as follows:



Thus, using endonucleases that are able to cleave after a G, A, C or a T in a given target nucleic acid sequence, fragments of the target nucleic acid are produced that end in each of these residues. Analysis of such fragments allows the specific identification and localization of the residues in that given sequence. Thus, the methods of the present invention provide a mass-spectrometry-based alternative to methods of sequencing e.g., they provide an alternative to conventional Sanger sequencing or conventional sequencing by hybridization techniques. This is different from the technique of Koster, which only teaches methods of detection of a sequence and not methods of iteratively deducing the identity of the sequence of a target nucleic acid by cleaving the target nucleic acid in two or more cleavage (i.e., multiple) reactions where each cleavage reaction generates a non-ordered set fragments.

As the two methods are different, Koster cannot anticipate the claims of the present invention. At no point anywhere in Koster is it suggested that multiple cleavage reactions that generate non-ordered fragments can be used to deduce the sequence of a given target nucleic acid. As this is a required step of each of claims 57 and 96, Applicants submit that these claims are novel over Koster.

In view of the above discussion, Applicants submit that all of the rejections of the claims of the instant application are overcome. Applicants respectfully request that the rejections be withdrawn and the claims be reconsidered for allowance.

III. Rejections of the Methods Claims Under 35 U.S.C. §103 Should be Withdrawn

Claims 58-71, 73-80, 82-83, 85-91 and 105-107 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over a combination of the disclosure of Koster (PCT International Publication No. WO 96/29431; Sept. 26, 1996) in view of Monforte et al. (PCT International Publication No. WO 97/33000). Applicants respectfully disagree. The Examiner indicates that Koster is being applied to claims 57 and 96 and is being supplemented by Monforte, which is alleged to provide a teaching of the features cited in the dependent claims. Applicants respectfully submit that the rejection as articulated by the Examiner between pages 4 and 9 of the office action fails to establish a *prima facie* case of obviousness.

As indicated above, the method of Koster simply fails to teach sequencing or re-sequencing using mass spectrometry as claimed in the present application. Thus, regardless of what the secondary reference (Monforte) might teach, unless it provides a specific teaching of ***sequencing of a target nucleic acid***, it is incapable of rehabilitating Koster for the purposes of rendering obvious the claims presented herein. As discussed in the previous response submitted July 23, 2003, Monforte simply fails to achieve this. To briefly reiterate, Monforte uses nonrandom fragmentation technique(s) for the ***detection*** of sequence variations in a target nucleic acid relative to a reference (*i.e.* wild type) nucleic acid. Monforte expressly states that the methods described in that publication ***do not*** involve sequencing of a target nucleic acid. For example, Applicants refer to page 10, lines 2-6,

where Monforte begins the Summary of their invention by explicitly disclaiming that the invention described therein:

> "provides methods of and kits for detecting mutations in a target nucleic acid comprising nonrandomly fragmenting said target nucleic acid to form a set of nonrandom length fragments (NLFs), determining the masses of members of said set of NLFs using mass spectrometry, ***wherein said determining does not involve sequencing of said target nucleic acid.*** " ***(Emphases added)***

As ***there is no indication*** that any of the mass spectrometry data generated by Monforte's methods are used to compile the sequence of the target, or to recompile the initial target sequence, Monforte fails to rehabilitate the disclosure of Koster.

Both the methods of Monforte and the methods of Koster only provide some limited information "about the nature and location of the mutation in the target nucleic acid" (page 16, lines 24-25) because a shift in the mass of an NLF of Monforte is indicative of the presence of a mutation. However, this information is indeed limited because, at best, using this information one can only "localize the region containing the mutation" by using overlapping fragments (page 35, lines 20-30). Thus, while it might be possible to use Monforte or Koster to show that there is "a difference" between a wild-type and a mutant sequence, it ***would not*** be possible to determine the identity of the sequence. Thus, the combination of Monforte and Koster is flawed because it fails to render obvious the subject matter of independent claims 57 and 96, let alone any of the claims dependent thereon.

Moreover, the combined disclosures of Koster and Monforte, counters the Examiner's contention that the combination renders obvious the claimed invention because there are explicit disclosures in that combination that states that the methods "***do[es] not involve sequencing of said target nucleic acid.***" (see Monforte at page 10, lines 2-6). Nothing in Koster refutes this statement from Monforte, and Koster itself is only directed to methods of detection, rather than methods of sequencing. As such, Applicants submit that the combination on its face states that sequencing of target nucleic acids is not contemplated. Moreover, given this statement in the Monforte document, one of skill in the art would not have been motivated to perform any kind of experimentation, routine or otherwise, to determine whether the methods of Koster could be modified in any way to render them

applicable for sequencing of a target nucleic acid. Again, both Koster and Monforte are directed ***only*** to ***detecting the presence or absence of a mutation*** (e.g., a change in sequence) and not to providing the sequence identity.

The claims of the present invention, in contrast to the cited art, are directed to sequencing a given target nucleic acid (e.g., claim 57), or re-sequencing a nucleic acid to confirm its sequence (*e.g.*, claim 89). In both of these aspects, the nucleotide sequence of the target nucleic acid is determined. These techniques provide an alternative to the conventional sequencing techniques available in the art. The techniques of the present invention use a "combination of several different mass spectra, obtained after complementary digestion reactions" (page 4, lines 28-29). Thus, in the presently claimed invention, the products of multiple cleavage reactions of a target nucleic acid are subjected to mass spectrometry Subsequently, the information from the various mass spectra is integrated to result in an unambiguous sequence determination, *i.e.* the combination of mass shifts and/or changes in cleavage pattern observed in the mass spectra results in the unequivocal determination of the sequence, including any variation from a known reference sequence where relevant. The only motivation or suggestion to use multiple cleavage reactions with endonucleases and to perform mass spectrometry analyses of the fragments for sequencing of a target nucleic acid arises out of the instant application and it is impermissible to use the Applicants' own disclosure to find a motivation for the claimed invention, as this would be hindsight reconstruction.

Nothing in the Koster/Monforte combination would suggest or motivate the skilled artisan to perform sequencing in the manner disclosed and claimed in the present application. In the absence of such a suggestion or motivation, the combination recited by the Examiner fails satisfy a key requirement for establishing obviousness because the only suggestion or motivation to perform such steps is found in the Applicants' own disclosure and not in the prior art. Given that one of skill in the art would not have been motivated to perform the methods of nucleic acid sequencing as described in the present application, there can be no expectation of successfully arriving at the claimed methods. For these reasons alone, Applicants respectfully submit that the articulated rejection fails to establish *prima facie* case of obviousness under 35 35 U.S.C. §103(a) over Koster in view of Monforte.

Moreover, there also are further reasons as to why the combination of Koster and Monforte fails to render obvious the claimed methods. With regard to rejections under 35 U.S.C. §103(a), it is incumbent upon the Examiner to provide evidence that, ***as a whole, shows that the legal determination sought to be proved*** (i.e., the reference teachings establishing a *prima facie* case of obviousness) is more probable than not. It is well established that in order to rely on a reference as a basis for rejection of an Applicant's invention, the reference must either be in the field of Applicant's endeavor or, if not, then be reasonably pertinent to the particular problem with which the inventor was concerned. In re Oetiker, 977 F.2d 1443, 1446, 24 USPQ2d 1443, 1445 (Fed. Cir. 1992). In the present case, the Examiner has sought to combine two references that are from art that is non-analogous to the field of the invention.

The field of endeavor of the presently claimed invention is the ***sequencing*** of target nucleic acids from a given source. The Examiner has relied on Monforte in combination with the Koster to assert obviousness of the instant claims. However, the field of endeavor of both of the cited references is ***detection*** of a nucleic acid sequence ***without sequencing of the target nucleic acid.*** These two fields of endeavor are not analogous. In the present invention the methods provide an unambiguous determination of the target nucleic acid sequence, whereas in the cited art there is merely a qualitative representation of whether a mutation is present or absent.

Applicants submit that the combination of Koster and Monforte can only be arrived at through the use of the Applicants' own disclosure as a source of motivation to identify disparate, and unconnected disclosures in the art. Not only is this an impermissible hindsight construction of an obviousness rejection, it actually fails to reconstruct the invention because it wholly fails to supply a teaching of sequencing of the target nucleic acid. The mere fact that the reference can be modified is not sufficient to establish a *prima facie* case of obviousness. *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990) (see MPEP 2143.01).

The combination of Koster and Monforte is further combined by the Examiner with Geysen et al. (U.S. Patent No. 6,475,807) in support of a rejection of claim 72. Geysen is only cited for the proposition that the nucleotides contain/incorporate isotopes. Geysen teaches nothing about sequencing of a target nucleic acid that would rehabilitate the

combination of Koster and Monforte. As such, at least for the reasons given above, the combination of Koster/Monforte/Geysen fails to render obvious claim 72.

Koster is also combined with Hanna (U.S. Patent No. 6,107,039) to allegedly render obvious the subject matter of claim 81, which is a claim dependent from claim 57 that further provides that "the one or more target nucleic acids are phosphorothioate-modified single-stranded DNA or RNA, and wherein the cleavage reactions are performed with the nuclease P1." Again, Applicants respectfully disagree with the Examiner's assessment of the obviousness of this claim. As discussed above, Koster fails to teach sequencing of a target nucleic acid. At best, all Koster teaches how to detect the presence of a mutation by hybridizing the target to a reference nucleic acid and using an endonuclease to cleave the duplex formed. If the duplex contains a mismatch due to the presence of a mutation in the target, part of the duplex will be single stranded and readily cleaved by a single-strand endonuclease. Hanna merely describes one such nuclease (i.e., nuclease P1). Thus, at best, the combination of Koster and Hanna provides a method of detecting the presence of e.g., a mutation using nuclease P1. The combination still fails to teach sequencing and in the absence of such a teaching, the combined references cannot render obvious the subject matter of any of the claims of the present invention. The same reasoning applies to the rejection of claims 84 and 104 over a combination of Koster and the New England Biolabs Catalog. The catalog merely teaches T7 and SP6 polymerases, *i.e.*, products that can be used in the detection methods of Koster. The catalog provides no additional disclosure that can teach modification of the Koster detection method to produce a method of *de novo* sequencing or re-sequencing as claimed in the present application. As such, the combination of Koster with the New England Biolabs Catalog is flawed for much the same reasons as any of the other combinations discussed above.

In summary, Applicants submit that the combination of cited references fails to render obvious the subject matter of Claims 57 and 96 as there is no motivation or suggestion to combine the teachings of the cited references and these references are from non-analogous arts. Even if one skilled in the art were aware of both references, that individual would not have any expectation of success of achieving the claimed invention because that individual would not consider the secondary reference reasonably pertinent to the field of sequencing using a non-ordered set of fragments, seeing as it is expressly stated in

one of the references (Monforte) expressly states that the methods disclosed therein do not involve sequencing, and the other reference (Koster) is silent about sequencing and only discusses detection of a nucleic acid. The references therefore cannot establish the alleged *prima facie* obviousness of the invention of claims 57 and 96. Claims 58-95 and 97-99 all ultimately depend from one of these claims and are thus also patentable over the cited references.

In view of the foregoing, Applicants respectfully request that the rejections of methods claims 58-99 under 35 U.S.C. §103(a) over the combination of Koster with various referenced be withdrawn.

IV. Kit claims

Moving now to the claims directed to the kits of the present invention, namely claims 100, 102, and 108-114.

Claims 100 and 102 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by the disclosure at page 74 of the New England BioLabs Catalog (Product No. 203S and 203L, 1996-1997). Applicants respectfully disagree with this rejection. Claim 100 expressly requires that the kit comprise "a computer software for comparing the mass spectra of the one or more target nucleic acid with the mass spectra of the reference nucleic acid and deducing therefrom the nucleic acid sequence of the target nucleic acid" and claim 102 expressly requires that the kit contain " computer software for analysing the mass spectra of the sequence of said target nucleic acid resulting in one or more unique sequences." Applicants have reviewed the disclosure of the New England BioLabs Catalog and can find no indication of a kit that contains computer software for analysis of mass spectra. As such, as a matter of law the disclosure in New England BioLabs Catalog cited by the Examiner cannot anticipate claims 100 and 102. Applicants request that the rejection be withdrawn.

Claims 108-114 were rejected under 35 U.S.C. §103(a) over New England BioLabs Catalog (Product No. 203S and 203L, 1996-1997), in view of Monforte et al. (PCT International Publication Number WO 97/33000, September 12, 1997). The Examiner indicates that Monforte discloses "a computer software for comparing the mass spectra of the one or more target nucleic acid with the mass spectra of the reference nucleic acid and

deducing therefrom the nucleic acid sequence of the target nucleic acid." (page 14 of Office action). Applicants respectfully disagree with the Examiner. Monforte explicitly disclaims that it does not provide a teaching of nucleic acid sequencing. The various shortcomings of Monforte with respect to its lack of a teaching sequencing of a target nucleic acid from a non-ordered set of fragments have been discussed above. Nothing in the New England BioLabs Catalog overcomes those teachings to provide a kit for nucleic acid sequencing and therefore, Applicants respectfully request that the Examiner reconsider the rejection of the kit claims 100, 102, and 108-114.

V. Request for Interview

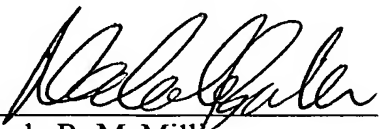
If upon review of the above amendments and discussion, the Examiner determines that a discussion with Applicants representative would facilitate a resolution of the outstanding issue, Applicants respectfully request the Examiner to contact the undersigned representative for a telephonic interview.

VI. Conclusions

Applicants believe that all of the rejections have been overcome and the claims of the instant application are now in condition for allowance and request an early indication of such a favorable disposition of the case. The Examiner is invited to contact the undersigned with any questions, comments or suggestions relating to the referenced patent application.

Dated: March 26, 2004

Respectfully submitted,

By 

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